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(54) Title: NOVEL PEPTIDES SUITABLE FOR USE IN ANTIGEN SPECIFIC IMMUNOSUPPRESSIVE THERAPY

(57) Abstract

The invention relates to peptides consisting of 16 to 55 amino acid residues, said peptides comprising at least one of the amino acid sequences LVCYYTSWS (SEQ ID NO:60), FLCTHIIS (SEQ ID NO:61), IIYSFANIS (SEQ ID NO:62), LKTLLSVGG (SEQ ID NO:63), FIKSVPPFL (SEQ ID NO:64), FDGLDLAWL (SEQ ID NO:65), LYPGRRDKQ (SEQ ID NO:66), YDIAKISQH (SEQ ID NO:67), LDFISIMTY (SEQ ID NO:68), FISIMTYDF (SEQ ID NO:69), FRGQEDASP (SEQ ID NO:70), YAVGYMLRL (SEQ ID NO:71), MLRLGAPAS (SEQ ID NO:72), LAYYEICDF (SEQ ID NO:73), LRGATVHRT (SEQ ID NO:74), YLKDRQLAG (SEQ ID NO:75), LAGAMVWAL (SEQ ID NO:76), VVALDDDF (SEQ ID NO:77) or LDLDDFQGS (SEQ ID NO:78). The peptides can be used in the treatment of T-cell mediated destruction of articular cartilage. Administration of pharmaceutical compositions based on these peptides can be used to induce systemic immunological tolerance to the autoantigens under attack of the autoreactive T-cells.

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NOVEL PEPTIDES SUITABLE FOR USE IN ANTIGEN SPECIFIC
IMMUNOSUPPRESSIVE THERAPY

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The invention relates to peptides and their use in treatment of chronic destruction of articular cartilage in autoimmune diseases, pharmaceutical compositions comprising said peptides, a diagnostic method for the detection of autoreactive T cells in 10 a test sample and test kits to be used in said method.

The immune system is established on a principle of discrimination between foreign antigens (non-self antigens) and autoantigens (self antigens, derived from the individuals own 15 body) achieved by a build in tolerance against the autoantigens.

The immune system protects individuals against foreign antigens and responds to exposure to a foreign antigen by activating specific cells such as T- and B lymphocytes and producing soluble factors like interleukins, antibodies and 20 complement factors. The antigen to which the immune system responds is degraded by the antigen presenting cells (APCs) and a fragment of the antigen is expressed on the cell surface associated with a major histocompatibility complex (MHC) class II glycoprotein. The MHC-glycoprotein-antigen-fragment complex 25 is presented to a T cell which by virtue of its T cell receptor recognizes the antigen fragment conjointly with the MHC class II protein to which it is bound. The T cell becomes activated, i.e. proliferates and/or produces interleukines, resulting in the expansion of the activated lymphocytes directed to the antigen 30 under attack (Grey et al., Sci. Am., 261:38-46, 1989).

Self antigens are also continuously processed and presented as antigen fragments by the MHC glycoproteins to T cells (Jardetsky et al., Nature 353:326-329, 1991). Self recognition

thus is intrinsic to the immune system. Under normal circumstances the immune system is tolerant to self antigens and activation of the immune response by these self antigens is avoided.

5 When tolerance to self antigens is lost, the immune system becomes activated against one or more self antigens, resulting in the activation of autoreactive T cells and the production of autoantibodies. This phenomenon is referred to as autoimmunity. As the immune response in general is destructive, i.e. meant to
10 destroy the invasive foreign antigen, autoimmune responses can cause destruction of the body's own tissue.

The contribution of T cells to autoimmune diseases has been established by several studies. In mice, experimental autoimmune encephalomyelitis (EAE) is mediated by a highly restricted group of T cells, linked by their specificity for a single epitope of myelin basic protein (MBP) complexed to an MHC class II molecule. In the Lewis rat, a species with high susceptibility to various autoimmune diseases, disease has been
20 shown to be mediated by T cells.

In humans autoimmune diseases are also thought to be associated with the development of auto-aggressive T cells. A destructive autoimmune response has been implicated in various diseases such as rheumatoid arthritis (RA), in which the integrity of articular cartilage is destroyed by a chronic inflammatory process. The mere presence of cartilage appears necessary for sustaining the local inflammatory response: it has been shown that cartilage degradation is associated with the activity of cartilage-responsive autoreactive T cells in RA
25 (Sigall et al., Clin. Exp. Rheumat. 6:59, 1988; Glant et al., Biochem. Soc. Trans. 18:796, 1990; Burmester et al., Rheumatoid arthritis Smolen, Kalden, Maini (Eds) Springer-Verlag Berlin
30

Heidelberg, 1992). Furthermore, removal of cartilage from RA patients by surgery was shown to reduce the inflammatory process. The cartilage proteins are therefore considered to be target autoantigens which are competent of stimulating T cells.

5 Activation of these autoreactive T cells leads to development of autoimmune disease. Hence it can be anticipated that functional elimination of these T cells could be beneficial in downregulation of the destructive autoimmune process. However, the identification of the autoantigenic components that play a

10 role in the onset of rheumatoid arthritis has so far remained elusive.

The inflammatory response resulting in the destruction of the cartilage can be treated by various drugs. However, these

15 drugs are immunosuppressive drugs that are nonspecific and have toxic side effects. The disadvantages of nonspecific immunosuppression makes this a highly unfavourable therapy.

Antigen-specific, nontoxic immunosuppression, such as for instance described in WO-A-9510301, provides a very attractive

20 alternative for nonspecific immunosuppression. The antigen-specific therapy involves the treatment of patients with synthetic T cell-reactive peptides which resemble or mimic the epitopes present on the autoantigen. These peptides can therefore be used to induce systemic immunological tolerance,

25 i.e. specific T cell tolerance, both to themselves and to the autoantigen. The induced systemic immunological tolerance is based on the long-observed phenomenon that animals which have been fed or have inhaled an antigen or epitope are less capable of developing a systemic immune response towards said antigen or

30 epitope when said antigen or epitope is introduced via a systemic route. To effectively use the peptide-induced systemic tolerance therapy to treat the T cell mediated cartilage

destruction, there is a great need for T cell-reactive peptides which can desensitize patients against the self antigen that is activating the T cells responsible for the inflammatory process.

5 It is an object of the invention to provide peptides which are able to induce systemic immunological tolerance, more in particular specific T cell tolerance, to the responsible cartilage antigen in patients suffering from T cell-mediated cartilage destruction. It is another object of the invention to
10 provide a method for detecting autoreactive T cells involved in the destruction of articular cartilage and test kits to be used in said method.

15 The present invention provides for such peptides.

In a first aspect of the invention there is provided for peptides consisting of 16 to 55 amino acid residues, said peptide comprising at least one of the amino acid sequences LVCYYTSWS (SEQ ID NO:60), FLCTHIIYS (SEQ ID NO:61), IIYSFANIS 20 (SEQ ID NO:62), LKTLLSVGG (SEQ ID NO:63), FIKSVPPFL (SEQ ID NO:64), FDGLDLAWL (SEQ ID NO: 65), LYPGRRDKQ (SEQ ID NO:66), YDIAKISQH (SEQ ID NO:67), LDFISIMTY (SEQ ID NO:68), FISIMTYDF (SEQ ID NO:69), FRGQEDASP (SEQ ID NO:70), YAVGYMLRL (SEQ ID NO:71), MLRLGAPAS (SEQ ID NO:72), LAYYEICDF (SEQ ID NO:73),
25 LRGATVHRT (SEQ ID NO:74), YLKDRQLAG (SEQ ID NO:75), LAGAMVWAL (SEQ ID NO:76), VWALDLDDF (SEQ ID NO:77) or LDLDFFQGS (SEQ ID NO:78).

In particular, the peptide according to the invention comprises at least one of the amino acid sequences
30 YKLVCYYTSWSQYREG (SEQ ID NO:1), YTWSQYREGDGSCFP (SEQ ID NO:2), LDRFLCTHIIYSFANI (SEQ ID NO:5), THIIYSFANISNDHID (SEQ ID NO:6), PNLKTLLSVGGWNFGS (SEQ ID NO:12), NTQSRRTFIKSVPPFL (SEQ ID

NO:16), TFIKSVPPFLRTHGFD (SEQ ID NO:17), PPFLRTHGFDGLDLAW (SEQ ID NO:18), HGF DGLDLAWLYPGRR (SEQ ID NO:19), DLAWLYPGRRDKQHFT (SEQ ID NO:20), TIDSSYDIAKISQHLD (SEQ ID NO:28), DIAKISQHLD FISIMT (SEQ ID NO:29), QHLD FISIMTYDFHGA (SEQ ID NO:30), SPLFRGQEDASPDRFS (SEQ ID NO:34), DYAVGYMLRLGAPASK (SEQ ID NO:37), MLRLGAPASKLVMGIP (SEQ ID NO:38), PASKLVMGIPTFGRSF (SEQ ID NO:39), GTLAYYEICDFLRGAT (SEQ ID NO:46), EICDFLRGATVHRTLG (SEQ ID NO:47), RGATVHRTLGQQVPYA (SEQ ID NO:48), VKSKVQYLKDRQLAGA (SEQ ID NO:53), YLKDRQLAGAMVWALD (SEQ ID NO:54), LAGAMVWALDLDDFQG (SEQ ID NO:55), WALDLDDFQGSFCGQD (SEQ ID NO:56) or DFQGSFCGQDLRFPLT (SEQ ID NO:57).

Preferably, the peptide according to the present invention comprises one of the amino acid sequences YKLVCYYTSWSQYREG (SEQ ID NO:1), YTSWSQYREGDGSCFP (SEQ ID NO:2), LDRFLCTHIIYSFANI (SEQ ID NO:5), THIIYSFANISNDHID (SEQ ID NO:6), PNLKTLLSVGGWNFGS (SEQ ID NO:12), QHLD FISIMTYDFHGA (SEQ ID NO:30), SPLFRGQEDASPDRFS (SEQ ID NO:34), DYAVGYMLRLGAPASK (SEQ ID NO:37), MLRLGAPASKLVMGIP (SEQ ID NO:38), YLKDRQLAGAMVWALD (SEQ ID NO:54) or LAGAMVWALDLDDFQG (SEQ ID NO:55).

More preferably, the peptide according to the invention comprises one or more of the amino acid sequences YTSWSQYREGDGSCFP (SEQ ID NO:2), SPLFRGQEDASPDRFS (SEQ ID NO:34), MLRLGAPASKLVMGIP (SEQ ID NO:38), YLKDRQLAGAMVWALD (SEQ ID NO:54) or LAGAMVWALDLDDFQG (SEQ ID NO:55).

The peptides according to the invention consist of 16 to 55, preferably 16 to 35, more preferably 16 to 25, most preferably 16 amino acid residues.

Highly preferred peptides according to the invention are hexadecapeptides consisting of the amino acid sequence YKLVCYYTSWSQYREG (SEQ ID NO:1) YTSWSQYREGDGSCFP (SEQ ID NO:2), LDRFLCTHIIYSFANI (SEQ ID NO:5), THIIYSFANISNDHID (SEQ ID NO:6), PNLKTLLSVGGWNFGS (SEQ ID NO:12), QHLD FISIMTYDFHGA (SEQ ID NO:30).

NO:30), SPLFRGQEDASPDRFS (SEQ ID NO:34), DYAVGYMLRLGAPASK (SEQ ID NO:37), MLRLGAPASKLVMGIP (SEQ ID NO:38), YLKDRQLAGAMVWALD (SEQ ID NO:54) or LAGAMVWALDLDDFQG (SEQ ID NO:55), more in particular the amino acid sequences YTSWSQYREGDGSCFP (SEQ ID NO:2), SPLFRGQEDASPDRFS (SEQ ID NO:34), MLRLGAPASKLVMGIP (SEQ ID NO:38), YLKDRQLAGAMVWALD (SEQ ID NO:54) or LAGAMVWALDLDDFQG (SEQ ID NO:55).

Also within the scope of the invention are multimers of the peptides according to the invention such as for example a dimer 10 or trimer of the peptides according to the invention. A multimer according to the invention can either be a homomer, consisting of a multitude of the same peptide, or a heteromer consisting of different peptides.

The characteristic amino acid sequences of the peptides 15 according to the invention can be flanked by random amino acid sequences. Preferred are flanking sequences, that have a stabilizing effect on the peptides, thus increasing their biological availability.

20 The present invention is based on the unexpected discovery, that Human Cartilage glycoprotein 39 (herein after referred to as HC gp-39) is a target autoantigen in RA patients which activates specific T cells, thus causing or mediating the inflammatory process. HC gp-39 derived peptides were 25 predominantly recognized by autoreactive T cells from RA patients but rarely by T cells from healthy donors, thus indicating that HC gp-39 is an autoantigen in RA. The arthritogenic nature of HC gp-39 was further substantiated in the Balb/c mouse. A single, subcutaneous injection of said 30 protein in Balb/c mice was able to initiate arthritic signs in the animals. The course of the HC gp-39- induced disease was characterized by relapses occurring periodically in fore paws

and/or hind paws and gradually developed from a mild arthritis into a more severe form. Also, a symmetrical distribution of afflicted joints was observed which is, together with the observation of recurrent relapses and nodule formation, 5 reminiscent of disease progression in arthritis, especially RA.

Even more surprisingly it was found that administration of HC gp-39 resulted in immunological tolerance and, more importantly, in delayed and/or suppressed arthritic development.

The amino acid sequences given in SEQ ID NO's 60-78, more 10 specifically the sequences given in SEQ ID NO's 1, 2, 5, 6, 12, 16-20, 28-30, 34, 37-39, 46-48, 53-57 resemble MHC class II restricted T cell epitopes which are present on HC gp-39. Thus, the peptides according to the invention can also be understood to encompass fragments of the autoantigen HC gp-39 which 15 comprise one or more of the above identified MHC Class II restricted T-cell epitopes and they are also within the scope of the invention.

Although HC gp-39 was disclosed in Hakala et al., J.Biol.Chem., Vol.268, No. 34, 25803 (1993), in which it was 20 described as a chitinase protein and suggested for use as a suitable marker for rheumatoid arthritis, any hint or suggestion towards the arthritogenic nature of HC gp-39 was absent.

The peptides according to the invention can be prepared by 25 well known organic chemical methods for peptide synthesis such as, for example, solid-phase peptide synthesis described for instance in J. Amer. Chem. Soc. 85:2149 (1963) and Int. J. Peptide Protein Res. 35:161-214 (1990).

The peptides according to the invention can also be prepared 30 by recombinant DNA techniques. A nucleic acid sequence coding for a peptide according to the invention or a multimer of said peptides is inserted into an expression vector. Suitable

expression vectors are, amongst others, plasmids, cosmids, viruses and YAC's (Yeast Artificial Chromosomes) which comprise the necessary control regions for replication and expression. The expression vector can be brought to expression in a host 5 cell. Suitable host cells are, for instance, bacteria, yeast cells and mammalian cells. Such techniques are well known in the art, see for instance Sambrooke et al, Molecular Cloning:a Laboratory Manual, Cold Spring Harbor laboratory Press, Cold Spring Harbor, 1989.

10

The peptides according to the invention are T-cell reactive peptides, which are recognized by and are able to stimulate activated, autoreactive T-cells. These autoreactive T cells are found in the blood of RA patients but rarely in healthy donors.

15

Thus, according to the invention the synthetic peptides, said peptides resembling the MHC Class II restricted T-cell epitopes present on the target autoantigen HC gp-39, are very suitable for use in a therapy to induce specific T-cell tolerance to HC gp-39 in mammals, more specifically humans, 20 suffering from T-cell mediated cartilage destruction, such as for example arthritis, more specifically rheumatoid arthritis.

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Although WO 95/01995 and WO 95/02188 describe the diagnostic use of HC gp-39 as a marker for RA, the arthritogenic nature of HC gp-39 is neither disclosed nor suggested. Nowhere do they hint or suggest towards the use of fragments of HC gp-39 or T-cell reactive peptides according to the present invention in the antigen or peptide specific therapy to induce T-cell specific tolerance to the HC gp-39 in the cartilage under attack.

30

According to the invention, patients suffering from T-cell mediated destruction of the articular cartilage can be treated with a therapeutical composition comprising one or more peptides

according to the invention and a pharmaceutical acceptable carrier. Administration of the pharmaceutical composition according to the invention will induce systemic immunological tolerance, in particular tolerance of the specific autoreactive 5 T cells of these patients, to the autoantigenic proteins in the articular cartilage under attack and other self antigens which display the identified MHC Class II binding T cell epitopes characterized or mimiced by the amino acid sequences of one or more of the peptides according to the invention. The induced 10 tolerance thus will lead to a reduction of the local inflammatory response in the articular cartilage under attack.

Very suitable peptides to be used in a pharmaceutical composition according to the invention are the peptides having 16-55, preferably 16-35, more preferably 16-25, most preferably 15 16 amino acid residues, said peptides comprising at least one of the amino acid sequences LVCYYTSWS (SEQ ID NO:60), FLCTHIIYS (SEQ ID NO:61), IIYSFANIS (SEQ ID NO:62), LKTLLSVGG (SEQ ID NO:63), FIKSVPPFL (SEQ ID NO:64), FDGLDLAWL (SEQ ID NO: 65), LYPGRRDKQ (SEQ ID NO:66), YDIAKISQH (SEQ ID NO:67), LDFISIMTY 20 (SEQ ID NO:68), FISIMTYDF (SEQ ID NO:69), FRGQEDASP (SEQ ID NO:70), YAVGYMLRL (SEQ ID NO:71), MLRLGAPAS (SEQ ID NO:72), LAYYEICDF (SEQ ID NO:73), LRGATVHRT (SEQ ID NO:74), YLKDRQLAG (SEQ ID NO:75), LAGAMVWAL (SEQ ID NO:76), VWALDLDDF (SEQ ID NO:77) or LDLDDFQGS (SEQ ID NO:78), more in particular one of 25 the amino acid sequences YKLVCYYTSWSQYREG (SEQ ID NO:1), YTSWSQYREGDGSCFP (SEQ ID NO:2), LDRFLCTHIIYSFANI (SEQ ID NO:5), THIIYSFANISNDHID (SEQ ID NO:6), PNLKTLLSVGGWNFGS (SEQ ID NO:12), NTQSRTFIKSVPPL (SEQ ID NO:16), TFIKSVPPLRTHGFD (SEQ ID NO:17), PPFLRTHGFDGLDLAW (SEQ ID NO:18), HGFGLDLAWLYPGRR (SEQ 30 ID NO:19), DLAWLYPGRRDKQHFT (SEQ ID NO:20), TIDSSYDIAKISQHLD (SEQ ID NO:28), DIAKISQHLD FISIMT (SEQ ID NO:29), QHLD FISIMTYDFHGA (SEQ ID NO:30), SPLFRGQEDASPDRFS (SEQ ID

NO:34), DYAVGYMLRLGAPASK (SEQ ID NO:37), MLRLGAPASKLVMGIP (SEQ ID NO:38), PASKLVMGIPTFGRSF (SEQ ID NO:39), GTLAYYEICDFLRGAT (SEQ ID NO:46), EICDFLRGATVHRTLG (SEQ ID NO:47), RGATVHRTLQQQVPA (SEQ ID NO:48), VKSKVQYLKDRQLAGA (SEQ ID NO:53), YLKDRQLAGAMVWALD (SEQ ID NO:54), LAGAMVWALDLDDFQG (SEQ ID NO:55), WALDLDDFQGSFCGQD (SEQ ID NO:56) or DFQGSFCGQDLRFPLT (SEQ ID NO:57).

Specifically preferred in a pharmaceutical composition according to the invention are the peptides having 16-55, preferably 16-35, more preferably 16-25, most preferably 16 amino acid residues, said peptides comprising at least one of the amino acid sequences YKLVCYYTSWSQYREG (SEQ ID NO:1), YTSWSQYREGDGSCFP (SEQ ID NO:2), LDRFLCTHIIYSFANI (SEQ ID NO:5), THIIYSFANISNDHID (SEQ ID NO:6), PNLKTLLSVGGWNFGS (SEQ ID NO:12), QHLDIFISIMTYDFHGA (SEQ ID NO:30), SPLFRGQEDASPDRFS (SEQ ID NO:34), DYAVGYMLRLGAPASK (SEQ ID NO:37), MLRLGAPASKLVMGIP (SEQ ID NO:38), YLKDRQLAGAMVWALD (SEQ ID NO:54) or LAGAMVWALDLDDFQG (SEQ ID NO:55).

Highly preferred in a pharmaceutical composition according to the invention are peptides having 16-55, preferably 16-35, more preferably 16-25, most preferably 16 amino acid residues, said peptides comprising at least one of the amino acid sequences YTSWSQYREGDGSCFP (SEQ ID NO:2), SPLFRGQEDASPDRFS (SEQ ID NO:34), MLRLGAPASKLVMGIP (SEQ ID NO:38), YLKDRQLAGAMVWALD (SEQ ID NO:54) or LAGAMVWALDLDDFQG (SEQ ID NO:55).

Most preferred in a pharmaceutical composition according to the invention are hexadecapeptides consisting of the amino acid sequence YKLVCYYTSWSQYREG (SEQ ID NO:1) YTSWSQYREGDGSCFP (SEQ ID NO:2), LDRFLCTHIIYSFANI (SEQ ID NO:5), THIIYSFANISNDHID (SEQ ID NO:6), PNLKTLLSVGGWNFGS (SEQ ID NO:12), QHLDIFISIMTYDFHGA (SEQ ID NO:30), SPLFRGQEDASPDRFS (SEQ ID NO:34), DYAVGYMLRLGAPASK (SEQ ID NO:37), MLRLGAPASKLVMGIP (SEQ ID NO:38), YLKDRQLAGAMVWALD

(SEQ ID NO:54) or LAGAMVWALDLDDFQG (SEQ ID NO:55), more in particular the amino acid sequences YTSWSQYREGDGSCFP (SEQ ID NO:2), SPLFRGQEDASPDRFS (SEQ ID NO:34), MLRLGAPASKLVMGIP (SEQ ID NO:38), YLKDRQLAGAMVWALD (SEQ ID NO:54) or LAGAMVWALDLDDFQG (SEQ 5 ID NO:55).

The peptides according to the invention have the advantage that they have a specific effect on the autoreactive T cells thus leaving the other components of the immune system intact as compared to the nonspecific suppressive effect of 10 immunosuppressive drugs. Treatment with the peptides according to the invention will be safe and no toxic side effects will occur.

Systemic immunological tolerance can be attained by 15 administering high or low doses of peptides according to the invention. The amount of peptide will depend on the route of administration, the time of administration, the age of the patient as well as general health conditions and diet.

In general, a dosage of 0.01 to 1000 µg of peptide per kg 20 body weight, preferably 0.5 to 500 µg, more preferably 0.1 to 100 µg of peptide can be used.

Pharmaceutical acceptable carriers are well known to those skilled in the art and include, for example, sterile salin, lactose, sucrose, calcium phosphate, gelatin, dextrin, agar, 25 pectin, peanut oil, olive oil, sesame oil and water. Other carriers may be, for example MHC class II molecules, if desired embedded in liposomes.

In addition the pharmaceutical composition according to the invention may comprise one or more adjuvants. Suitable adjuvants 30 include, amongst others, aluminium hydroxide, aluminium phosphate, amphigen, tocophenols, monophosphoryl lipid A, muramyl dipeptide and saponins such as Quill A. Preferably, the

adjuvants to be used in the tolerance therapy according to the invention are mucosal adjuvants such as the cholera toxine B-subunit or carboomers, which bind to the mucosal epithelium. The amount of adjuvant depends on the nature of the adjuvant itself.

5 Furthermore the pharmaceutical composition according to the invention may comprise one or more stabilizers such as, for example, carbohydrates including sorbitol, mannitol, starch, sucrosedextrin and glucose, proteins such as albumin or casein, and buffers like alkaline phosphates.

10 Suitable administration routes are intramuscular injections, subcutaneous injections, intravenous injections or intraperitoneal injections, oral administration and nasal sprays.

15 The peptides according to the invention are also very suitable for use in a diagnostic method to detect the presence of activated autoreactive T cells involved in the chronic inflammation and destruction of the articular cartilage.

The diagnostic method according to the invention comprises
20 the following steps:

- a) isolation of the peripheral blood mononuclear cells (PBMC) from a blood sample of an individual,
- b) culture said PBMC under suitable conditions,
- c) incubation of said PBMC culture in the presence of one or
25 more peptides according to the invention, and
- d) detection of a response of T cells, for example a proliferative response, indicating the presence of activated autoreactive T cells in the individual.

The detection of a proliferative response of T cells can be
30 detected by, for example, the incorporation of ^{3}H -thymidine.

Also within the scope of the invention are test kits which comprise one or more peptides according to the invention. These

test kits are suitable for use in a diagnostic method according to the invention.

The following examples are illustrative for the invention
5 and should in no way be interpreted as limiting the scope of the invention.

EXAMPLES

10 METHODS

Patients

This study included 7 DR4(DRB1*0401)-positive patients diagnosed as suffering from RA according to the ARA criteria
15 (Arnett et al., (1988), Arthritis Rheum. 31, 315). Peripheral blood samples were obtained with informed consent. There were five women and two men aged 46-79 years. Their duration of disease ranged from over 10 to over 30 years. Three out of 7 patients had at least 3 swollen joints. Four patients did not
20 show any signs of active disease. All patients were on medication: Four patients were treated with prednisone, three patients received anti-rheumatic agents and 4 patients were treated with NSAID's as well.

Peripheral blood samples from 5 healthy donors carrying the
25 DR4(DRB1*0401) specificity were obtained with informed consent and included in this study as a control.

Definition of HLA-DR polymorphisms

Patient and healthy donor peripheral blood mononuclear cells
30 (PBMC) isolated from heparinized peripheral blood by standard centrifugation on Ficoll-Paque were stimulated with PHA (Welcome, Dartford, UK) to obtain 5×10^6 - 10^7 lymphocytes. The

QIA amp blood kit (QIAGEN Inc,) was used to purify chromosomal DNA from cultured cells according to the manufacturers instructions. Chromosomal DNA extracts were analysed using a DR 'low resolution' SSP kit. DR4 subtyping was performed using the 5 Dynal DRB1*04-SSP kit. MHC DR typing was performed at the Transplant Serology Laboratory, University Hospital, Nijmegen, The Netherlands.

Table I

10

RA Patient	stage	synovitis	duration	HLA-DR
191	IV	no	>30 years	0401/01
259	III-IV	yes	>30 years	0401/16
262	III-IV	yes	>10 years	0401/0408
272	III-IV	no	> 30 years	0401/0701
276	IV	no	>30 years	0401/14
286	IV	no	20 years	0401/0408
287	III-IV	yes	20 years	0401/13
HD				HLA-DR
155				0401/14
157				0401/13
168				0401/07
230				0401/07
235				0401/13

Peptide synthesis

Peptides were synthesized at Eurosequence (Groningen, The 15 Netherlands). Peptides were synthesized from the C-terminus to the N-terminus on a 10 µmol scale using solid-phase FMOC

chemistry. The crude peptides were partly purified by several other preparations. As indicated by the manufacturer, at least 35% of the lyophilized product contained the desired full length product. The rest contained salt and remaining solvent. The quality of the final product was checked by sequence analysis, amino acid analysis and/or RF-HPLC. The sequences of the peptides synthesized are enlisted in Table II.

Table II: Amino acid sequences of the peptides used in this study.

SEQ ID NO:	residu	peptide
1	22-37	YKLVCVYTWSQYREG
2	28-43	YTSWSQYREGDGSCFP
3	34-49	YREGDGSCFPDALDRF
4	40-55	SCFPDALDRFLCTHII
5	46-61	LDRFLCTHIIYSFANI
6	52-67	THIIYSFANISNDHID
7	58-73	FANISNDHIDTWEWND
8	64-79	DHIDTWEWNDVTLYGM
9	70-85	EWNDVTLYGMLNTLKN
10	76-91	LYGMLNTLKNRNPNLK
11	82-97	TLKNRNPNLKTLSSVG
12	88-103	PNLKTLLSVGGWNFGS
13	94-109	LSVGGWNFGSQRFPSKI
14	100-115	NFGSQRFPSKIASNTQS
15	106-121	FSKIASNTQSRRRTFIK
16	112-127	NTQSRRTFIKSVPPFL
17	118-133	TFIKSVPFLRTHGFD
18	124-139	PPFLRTHGFDGLDLAW
19	130-145	HGFDGDLAWLYPGRR

20	136-151	DLAWLYPGRRDKQHFT
21	142-157	PGRRDKQHFTTLIKEM
22	148-163	QHFTTLIKEMKAEFIK
23	154-169	IKEMKAEFIKEAQPGK
24	160-175	EFIGEAQPGKKQLLLS
25	166-181	QPGKKQLLLSAALSAAG
26	172-187	LLSAALSAAGKVTIDS
27	178-193	LSAGKVTIDSSYDIAK
28	184-199	TIDSSYDIAKISQHLD
29	190-205	DIAKISQHLDIFISIMT
30	196-211	QHLDIFISIMTYDFHGA
31	202-217	SIMTYDFHGAWRGTTG
32	208-223	FHGAWRGTTGHHSPLF
33	214-229	GTTGHHSPLFRGQEDA
34	220-235	SPLFRGQEDASPDRFS
35	226-241	QEDASPDRFSNTDYAV
36	232-247	DRFSNTDYAVGYMLRL
37	238-253	DYAVGYMLRLGAPASK
38	244-259	MLRLGAPASKLVMGIP
39	250-265	PASKLVMGIPTFGRSF
40	256-271	MGIPTFGRSFTLASSE
41	262-277	GRSFTLASSETGVGAP
42	268-283	ASSETGVGAPISGPGI
43	274-289	VGAPISGPGIPGRFTK
44	280-295	GPGIPGRFTKEAGTLA
45	286-301	RFTKEAGTLAYYEICD
46	292-307	GTLAYYEICDFLRGAT
47	298-313	EICDFLRGATVHRTLG
48	304-319	RGATVHRTLGQQVPYA
49	310-325	RTLGGQQVPYATKGNQW
50	316-331	VPYATKGNQWVGYDDQ

51	322-337	GNQWVGYDDQESVKSK
52	328-343	YDDQESVKSKVQYLKD
53	334-349	VKSKVQYLKDRQLAGA
54	340-355	YLKDRQLAGAMVWALD
55	346-361	LAGAMVWALDLDDFQG
56	352-377	WALDLDDFQGSFCGQD
57	358-373	DFQGSFCGQDLRFPLT
58	364-379	CGQDLRFPLTNAIKDA
59	368-383	LRFPLTNAIKDALAAT

Peptide HLA-DR binding assay

DR4 (DRB1*0401) and DR4 (DRB1*0404) molecules were purified
5 from the homozygous EBV-transformed human B lymphoblastoid cell
lines Huly138IC2 and BM92 using the mAb L243, directed against a
monomorphic determinant on the DR-complex (Lampson, L.A. and R.
Levy (1980), J. Immunol. 125:293-299).

The peptide binding studies were performed using a semi-
10 quantitative competition binding assay (Joosten et al 1994,
Int. Immunol. 6, 751). Briefly, purified HLA-DR molecules (30 nM
DR4 (DRB1*0401) or 15 nM DR4 (DRB1*0404) were incubated at pH5.0
with 50 nM biotinylated marker peptide (HA 309_{Y→F}) and a
concentration range of competitor peptide in a final volume of
15 25 µl binding buffer (PBS containing 0.01% NaN₃, 0.05% NP-40, 5%
DMSO, 1 mM AEBSF, 1 mM N-ethyl maleimide, 8 mM EDTA and 10 µM
pepstatin A). After 44 hr of incubation at RT, HLA-DR-bound
marker peptide was separated from free marker peptide using a 96
well vacuum dot blot apparatus (Hybri.dot, BRL) and a
20 nitrocellulose membrane (Hybond ECL, Amersham, UK). The
nitrocellulose filters were blocked with 0.5% DNA blocking
reagent (Boehringer) in 0.1 M maleic acid, 150 mM NaCl, pH7.5.

After 0.5-1 hr, the filters were washed in PBS, 0.05% Tween 20 (Sigma) and incubated with Streptavidin-HRPO (Southern Biotechnology) in a 1:10.000 dilution. Biotinylated peptides were detected by enhanced chemiluminescence using a Western Blot ECL kit (Amersham). Exposure of the preflashed films (Hyperfilm-ECL, Amersham) was for 10 min. The spots were analysed by scanning the films and using Image Quant/Excel software for analysis.

The affinity of a given peptide for binding DRB1*0401-encoded molecules was related to competition with the marker peptide. This relative binding affinity was defined as the peptide concentration at which the signal was reduced to 50% (IC₅₀).

15 **Proliferative responses of blood mononuclear cells**

In order to identify T-cell epitopes within HC gp-39, 59 peptides of 16 AA in length, overlapping by 10 AA were tested for their capacity to induce a proliferative response in PBMC from RA patients and healthy controls carrying the DR4 (DRB1*0401) specificity (Table 1). Table 2 enlists the sequences of the peptides tested.

PBMC obtained from heparinized venous peripheral blood were isolated by standard centrifugation on a Ficoll-Paque gradient. Cells were cultured in four-fold at a concentration of 1,5 x 10⁵ cells / well in medium supplemented with 10% heat-inactivated, autologous plasma, L-glutamine, 2-ME and antibiotics in flatbottomed microtiter plates. Cells were incubated in medium alone or in the presence of phytohaemagglutinin (PHA) (2.5 µg/ml) to assert cell viability, or in the presence of 10 or 100 µg/ml of the HC gp-39-derived peptides. In several cases, sets of 2 or 3 sequential peptides were tested due to limited PBMC numbers of individual donors. Cultures were incubated in a total

volume of 210 µl for 7 days at 37 °C in a humidified atmosphere of 5% CO₂. Cultures were pulsed during the last 18 hours with 0.25 µCi [³H]-thymidine ([³H]TdR). Cells were harvested on glassfiber filters and [³H]TdR incorporation was measured by gas scintillation (Packard Matrix 96 βcounter). Only peptides inducing a proliferative response at both 10 and 100 µg/ml were considered to contain a T-cell epitope. Responses were defined positive if stimulation index values (SI, antigen-specific counts per 5min (cp5m) / background cp5m) exceeded or equaled 2.

10

RESULTS

Identification of T-cell epitopes by proliferative responses of 15 blood mononuclear cells

T-cell reactivity to HC gp-39-derived peptides was analyzed by measuring the PBMC proliferative response in DR4 (DRB1*0401)-positive RA patients and healthy donors. Proliferative responses were tested in autologous plasma. In Table IIIA and IIIB the results of 7 experiments are presented showing the responses of RA patients (Table IIIA) and the responses of healthy donors (Table IIIB) to 59 overlapping sequences derived from HC gp-39. Donors found to respond to both concentrations (100 and 10 µg/ml) of a peptide were ranked as responders and donors which 25 did not respond to both concentrations tested were ranked as non-responders (NR).

Responses to the individual peptides 1, 2, 5, 6, 12, 15, 30, 34, 37, 38, 40, 41, 54 and 55 (the numbers respond to the 30 respective SEQ ID NO of each peptide, for example, peptide 30 means: peptide having amino acid sequence of SEQ ID NO:30) were

observed in one or more donors, thereby identifying these sequences as T-cell epitopes.

Interestingly, responses to peptides 2, 34, 38, 40, 54 and 55 were observed in RA patients only.

5 On the other hand, peptides 12 and 41 induced only responses in healthy donors (230, 235) thus far.

In addition, as can be seen in Table 3, responses were found to the following sets of: peptides 1/2, 1/2/3, 4/5/6, 5/6, 10 15/16, 17/18, 19/20, 28/29/30, 29/30, 37/38, 37/38/39, 39/40, 46/47/48, 53/54, 55/56 and 55/56/57. These results are in accordance with most of the results of the individual peptides mentioned above. Moreover, the responses against the sets of peptides define regions that contain additional T-cell epitopes, i.e. the regions covered by peptides 16-20 (residu 112-151), 15 20 28-29 (residu 184-205), 38-40 (residu 244-271), 46-48 (residu 292-319) and 53-57 (334-373).

Six out of 7 DR4 (DRB1*0401)-positive RA patients responded to HC gp-39-derived peptides or sets of peptides and were 20 therefore ranked as responders. In the healthy donor group (HD), 3 out of 5 donors were ranked as responders. In general, RA patients appeared to respond to many more HC gp-39 regions than healthy donors (healthy donor 230 being an exception). For example, PBMC from RA patient 272, which were tested against 25 individual peptides, appeared to respond to a total of 11 peptides (1, 2, 5, 6, 30, 34, 37, 38, 40, 54 and 55). PBMC of the other patients (patient 287 being an exception) showed 30 responses against sets of peptides overlapping these 11 sequences and identified some additonal regions containing T-cell epitopes (peptides 14-20 and 46-48).

42									
43									
44									
45									
46								+++	
47						pos		+++	
48								+	
49									
50									
51&									
52&									
53								+++	
54	pos		pos	pos				+++	
55	pos			pos	pos			+++	
56								+++	
57								++	
58									
59									
BG	0.2	0.7	0.5	0.8	2.4	0.9	0.2		

pos = positive responses to both 100 and 10 microgram/ml of peptide or sets of peptides ($SI \geq 2$ were regarded positive). Together the peptides (16 AA in length and overlapping by 10 AA) cover the complete mature sequence of mature HC gp-39 (residu 22-383). Peptides were synthesized at Eurosequence (Groningen, The Netherlands). RA = rheumatoid arthritis patient. 0401 = donor carrying the RA-associated HLA-DRB1*0401 specificity. NR = non-responder. R = responder. BG = mean of background counts per 5 minutes $\times 10^{-3}$ measured in wells without antigen. +++ = high affinity binder ($IC50 < 1 \mu M$); ++ = good affinity binder ($1 < IC50 < 10 \mu M$); + = intermediate binder ($10 < IC50 < 100 \mu M$); +/- = poor binder ($100 < IC50 < 1000 \mu M$); - = non-binder ($IC50 > 1000 \mu M$)

Table IIIB: Peptide-induced proliferative responses of PBMC from healthy donors.

5

HD	155	157	168	230	235	0401
peptide	0401	0401	0401	0401	0401	binding
1	R	NR	NR	R	R	
2						
3						
4						
5				pos		+++
6				pos		+++
7						
8						
9						
10						
11						
12				pos		+++
13						
14&						
15&				pos		+++
16						
17						
18						
19						
20						
21						
22						
23						
24						

25					
26					
27					
28					
29					
30			pos		+++
31					
32					
33					
34					
35					
36					
37	pos		pos	pos	+++
38					
39					
40					
41				pos	+++
42					
43					
44					
45					
46					
47					
48					
49					
50					
51					
52					
53					
54					
55					

56						
57						
58						
59						
BG	4,2	10,4	2,2	3,6	3,5	

pos = positive responses to both 100 and 10 microgram/ml of peptide or sets of peptides ($SI \geq 2$ were regarded positive). Together the peptides (16 AA in length and overlapping by 10 AA) cover the complete mature sequence of mature HC gp-39 (residu 22-383). Peptides were synthesized at Eurosequence (Groningen, The Netherlands). HD = healthy donor. 0401 = donor carrying the RA-associated HLA-DRB1*0401 specificity. NR = non-responder. R = responder. BG = mean of background counts per 5 minutes $\times 10^{-3}$ measured in wells without antigen. +++ = high affinity binder ($IC50 < 1 \mu M$); ++ = good affinity binder ($1 < IC50 < 10 \mu M$); + = intermediate binder ($10 < IC50 < 100 \mu M$); +/- = poor binder ($100 < IC50 < 1000 \mu M$); - = non-binder ($IC50 > 1000 \mu M$)

15 ABBREVIATIONS

AEBSF: 4-(2-AminoEthyl)-BenzeneSulfonyl Fluoride
 BB: binding buffer
 BCA: Bicinchoninic Acid
 BSA: bovine serum albumin
 DMSO: Dimethyl Sulfoxide
 ECL: Enhanced Chemiluminescence
 EDTA: EthyleneDiamine Tetra Acetic acid
 FACS: Fluorescence Activated Cell Sorter
 HLA: Human Leukocyte Antigens
 HPLC: High Pressure Liquid Chormatography
 HRP: Horse Radish Peroxidase
 MHC CLASS II: Major Histocompatibility Complex class II

NMR: Nuclear Magnetic Resonance
NP-40: Nonidet P-40
PBS: Phosphate Buffered Saline
PVDF: Polyvinylidene difluoride
5 RA: Rheumatoid Arthritis
SDS-PAGE: Sodium DodecylSulfate Polyacrylamide Gel
Electrophoresis

SEQUENCE LISTING

(1) GENERAL INFORMATION:

5

(i) APPLICANT:

- (A) NAME: Akzo Nobel N.V.
- (B) STREET: Velperweg 76
- (C) CITY: Arnhem
- 10 (E) COUNTRY: The Netherlands
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15

(ii) TITLE OF INVENTION: Novel Peptides Suitable For Use In
Antigen Specific Immunosuppressive Therapy

(iii) NUMBER OF SEQUENCES: 78

20

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- 25 (D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

30

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids

29

- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Tyr Lys Leu Val Cys Tyr Tyr Thr Ser Trp Ser Gln Tyr Arg Glu Gly
1 5 10 15
15

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

20

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

30

Tyr Thr Ser Trp Ser Gln Tyr Arg Glu Gly Asp Gly Ser Cys Phe Pro
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 3:

5 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: peptide

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Tyr Arg Glu Gly Asp Gly Ser Cys Phe Pro Asp Ala Leu Asp Arg Phe
1 5 10 15

20

(2) INFORMATION FOR SEQ ID NO: 4:

25 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

30

31

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Ser Cys Phe Pro Asp Ala Leu Asp Arg Phe Leu Cys Thr His Ile Ile
1 5 10 15

5

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

20

Leu Asp Arg Phe Leu Cys Thr His Ile Ile Tyr Ser Phe Ala Asn Ile
1 5 10 15

25 (2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

32

(ii) MOLECULE TYPE: peptide

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Thr His Ile Ile Tyr Ser Phe Ala Asn Ile Ser Asn Asp His Ile Asp

1 5 10 15

10

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

25 Phe Ala Asn Ile Ser Asn Asp His Ile Asp Thr Trp Glu Trp Asn Asp

1 5 10 15

30

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids

33

- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

10

Asp His Ile Asp Thr Trp Glu Trp Asn Asp Val Thr Leu Tyr Gly Met

1

5

10

15

15 (2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

20

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

30

Glu Trp Asn Asp Val Thr Leu Tyr Gly Met Leu Asn Thr Leu Lys Asn

1

5

10

15

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- 5 (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

15 Leu Tyr Gly Met Leu Asn Thr Leu Lys Asn Arg Asn Pro Asn Leu Lys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 11:

20

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- 25 (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

35

Thr Leu Lys Asn Arg Asn Pro Asn Leu Lys Thr Leu Leu Ser Val Gly
1 5 10 15

5 (2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- 10 (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Pro Asn Leu Lys Thr Leu Leu Ser Val Gly Gly Trp Asn Phe Gly Ser
20 1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 13:

25

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- 30 (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

36

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Leu Ser Val Gly Gly Trp Asn Phe Gly Ser Gln Arg Phe Ser Lys Ile

1

5

10

15

5

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 16 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

20

Asn Phe Gly Ser Gln Arg Phe Ser Lys Ile Ala Ser Asn Thr Gln Ser

1

5

10

15

25 (2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 16 amino acids

(B) TYPE: amino acid

30

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Phe Ser Lys Ile Ala Ser Asn Thr Gln Ser Arg Arg Thr Phe Ile Lys

1 5 10 15

10

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- 15 (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

20

(v) FRAGMENT TYPE: internal

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Asn Thr Gln Ser Arg Arg Thr Phe Ile Lys Ser Val Pro Pro Phe Leu

1 5 10 15

30

(2) INFORMATION FOR SEQ ID NO: 17:

38

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- 5 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

15 Thr Phe Ile Lys Ser Val Pro Pro Phe Leu Arg Thr His Gly Phe Asp

1

5

10

15

(2) INFORMATION FOR SEQ ID NO: 18:

20

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- 25 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

30

39

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Pro Pro Phe Leu Arg Thr His Gly Phe Asp Gly Leu Asp Leu Ala Trp
1 5 10 15

5

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

His Gly Phe Asp Gly Leu Asp Leu Ala Trp Leu Tyr Pro Gly Arg Arg
1 5 10 15

25

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

40

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

5 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

10

Asp Leu Ala Trp Leu Tyr Pro Gly Arg Arg Asp Lys Gln His Phe Thr
1 5 10 15

15 (2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16 amino acids

(B) TYPE: amino acid

20 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

25 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

30

Pro Gly Arg Arg Asp Lys Gln His Phe Thr Thr Leu Ile Lys Glu Met
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- 5 (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

10

(v) FRAGMENT TYPE: internal

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Gln	His	Phe	Thr	Thr	Leu	Ile	Lys	Glu	Met	Lys	Ala	Glu	Phe	Ile	Lys
1		5						10						15	

20

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- 25 (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

30

(v) FRAGMENT TYPE: internal

42

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

Ile Lys Glu Met Lys Ala Glu Phe Ile Lys Glu Ala Gln Pro Gly Lys
1 5 10 15
5

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Glu Phe Ile Lys Glu Ala Gln Pro Gly Lys Lys Gln Leu Leu Leu Ser
1 5 10 15
25

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

5 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

10

Gln Pro Gly Lys Lys Gln Leu Leu Leu Ser Ala Ala Leu Ser Ala Gly
1 5 10 15

15 (2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- 20 (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

25 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

30

Leu Leu Leu Ser Ala Ala Leu Ser Ala Gly Lys Val Thr Ile Asp Ser
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 16 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: peptide

 (v) FRAGMENT TYPE: internal

15

 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

Leu Ser Ala Gly Lys Val Thr Ile Asp Ser Ser Tyr Asp Ile Ala Lys

1 5 10 15

20

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 16 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: peptide

 (v) FRAGMENT TYPE: internal

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

Thr Ile Asp Ser Ser Tyr Asp Ile Ala Lys Ile Ser Gln His Leu Asp
1 5 10 15
5

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

Asp Ile Ala Lys Ile Ser Gln His Leu Asp Phe Ile Ser Ile Met Thr
1 5 10 15

25

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

5 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

10

Gln His Leu Asp Phe Ile Ser Ile Met Thr Tyr Asp Phe His Gly Ala

1

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10

15

15 (2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16 amino acids

(B) TYPE: amino acid

20 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

25 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

30

Ser Ile Met Thr Tyr Asp Phe His Gly Ala Trp Arg Gly Thr Thr Gly

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15

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- 5 (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

10

(v) FRAGMENT TYPE: internal

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

Phe His Gly Ala Trp Arg Gly Thr Thr Gly His His Ser Pro Leu Phe
1 5 10 15

20

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- 25 (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

30

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

Gly Thr Thr Gly His His Ser Pro Leu Phe Arg Gly Gln Glu Asp Ala

1

5

10

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5

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

Ser Pro Leu Phe Arg Gly Gln Glu Asp Ala Ser Pro Asp Arg Phe Ser

1

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25

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

5 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

10

Gln Glu Asp Ala Ser Pro Asp Arg Phe Ser Asn Thr Asp Tyr Ala Val

1

5

10

15

15 (2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16 amino acids

(B) TYPE: amino acid

20 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

25 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

30

Asp Arg Phe Ser Asn Thr Asp Tyr Ala Val Gly Tyr Met Leu Arg Leu

1

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50

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- 5 (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

10

(v) FRAGMENT TYPE: internal

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

Asp Tyr Ala Val Gly Tyr Met Leu Arg Leu Gly Ala Pro Ala Ser Lys
1 5 10 15

20

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- 25 (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

30

(v) FRAGMENT TYPE: internal

51

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

Met Leu Arg Leu Gly Ala Pro Ala Ser Lys Leu Val Met Gly Ile Pro
1 5 10 15

5

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

Pro Ala Ser Lys Leu Val Met Gly Ile Pro Thr Phe Gly Arg Ser Phe
1 5 10 15

25

(2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

52

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

5 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

10

Met Gly Ile Pro Thr Phe Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu

1

5

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15

15 (2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16 amino acids

(B) TYPE: amino acid

20

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

25

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

30

Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly Val Gly Ala Pro

1

5

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15

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:

5

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

Ala Ser Ser Glu Thr Gly Val Gly Ala Pro Ile Ser Gly Pro Gly Ile
1 5 10 15

20

(2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:

25

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

30

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

54

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

Val Gly Ala Pro Ile Ser Gly Pro Gly Ile Pro Gly Arg Phe Thr Lys

1 5 10 15

5

(2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 16 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

Gly Pro Gly Ile Pro Gly Arg Phe Thr Lys Glu Ala Gly Thr Leu Ala

1 5 10 15

25

(2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 16 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

55

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

5 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

10

Arg Phe Thr Lys Glu Ala Gly Thr Leu Ala Tyr Tyr Glu Ile Cys Asp
1 5 10 15

15 (2). INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16 amino acids

(B) TYPE: amino acid

20 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

25 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

30

Gly Thr Leu Ala Tyr Tyr Glu Ile Cys Asp Phe Leu Arg Gly Ala Thr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 47:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 16 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: peptide

 (v) FRAGMENT TYPE: internal

15

 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

Glu Ile Cys Asp Phe Leu Arg Gly Ala Thr Val His Arg Thr Leu Gly

1 5 10 15

20

(2) INFORMATION FOR SEQ ID NO: 48:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 16 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: peptide

 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

Arg Gly Ala Thr Val His Arg Thr Leu Gly Gln Gln Val Pro Tyr Ala
5 1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 49:

10

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

Arg Thr Leu Gly Gln Gln Val Pro Tyr Ala Thr Lys Gly Asn Gln Trp
25 1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 50:

30

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid

58

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

5

(v) FRAGMENT TYPE: internal

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

Val	Pro	Tyr	Ala	Thr	Lys	Gly	Asn	Gln	Trp	Val	Gly	Tyr	Asp	Asp	Gln
1				5						10				15	

15

(2) INFORMATION FOR SEQ ID NO: 51:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16 amino acids

20

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

25

(v) FRAGMENT TYPE: internal

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

59

Gly Asn Gln Trp Val Gly Tyr Asp Asp Gln Glu Ser Val Lys Ser Lys
1 5 10 15

5 (2) INFORMATION FOR SEQ ID NO: 52:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- 10 (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

15 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

20

Tyr Asp Asp Gln Glu Ser Val Lys Ser Lys Val Gln Tyr Leu Lys Asp
1 5 10 15

25 (2) INFORMATION FOR SEQ ID NO: 53:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- 30 (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

60

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

Val Lys Ser Lys Val Gln Tyr Leu Lys Asp Arg Gln Leu Ala Gly Ala
10 1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 54:

15 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

Tyr Leu Lys Asp Arg Glu Leu Ala Gly Ala Met Val Trp Ala Leu Asp
30 1 5 10 15

61

(2) INFORMATION FOR SEQ ID NO: 55:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 16 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

10

(v) FRAGMENT TYPE: internal

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

Leu Ala Gly Ala Met Val Trp Ala Leu Asp Leu Asp Asp Phe Gln Gly
1 5 10 15

20

(2) INFORMATION FOR SEQ ID NO: 56:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 16 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

30

(v) FRAGMENT TYPE: internal

62

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

Trp Ala Leu Asp Leu Asp Asp Phe Gln Gly Ser Phe Cys Gly Gln Asp

1 5 10 15

5

(2) INFORMATION FOR SEQ ID NO: 57:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

Asp Phe Gln Gly Ser Phe Cys Gly Gln Asp Leu Arg Phe Pro Leu Thr

1 5 10 15

25

(2) INFORMATION FOR SEQ ID NO: 58:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

5 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

10

Cys Gly Gln Asp Leu Arg Phe Pro Leu Thr Asn Ala Ile Lys Asp Ala
1 5 10 15

15 (2) INFORMATION FOR SEQ ID NO: 59:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- 20 (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

25 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:

30

Leu Arg Phe Pro Leu Thr Asn Ala Ile Lys Asp Ala Leu Ala Ala Thr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 60:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 9 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: peptide

 (v) FRAGMENT TYPE: internal

15

 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:

Leu Val Cys Tyr Tyr Thr Ser Trp Ser

1 5

20

(2) INFORMATION FOR SEQ ID NO: 61:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 9 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: peptide

 (v) FRAGMENT TYPE: internal

65

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

Phe Leu Cys Thr His Ile Ile Tyr Ser

1 5

5

(2) INFORMATION FOR SEQ ID NO: 62:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 62:

Ile Ile Tyr Ser Phe Ala Asn Ile Ser

1 5

25

(2) INFORMATION FOR SEQ ID NO: 63:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

66

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

5 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 63:

10

Leu Lys Thr Leu Leu Ser Val Gly Gly

1 5

15 (2) INFORMATION FOR SEQ ID NO: 64:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids

(B) TYPE: amino acid

20 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

25 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 64:

30

Phe Ile Lys Ser Val Pro Pro Phe Leu

1 5

(2) INFORMATION FOR SEQ ID NO: 65:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 9 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: peptide

 (v) FRAGMENT TYPE: internal

15

 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 65:

Phe Asp Gly Leu Asp Leu Ala Trp Leu

1 5

20

(2) INFORMATION FOR SEQ ID NO: 66:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 9 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: peptide

 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 66:

5

Leu Tyr Pro Gly Arg Arg Asp Lys Gln

1

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10 (2) INFORMATION FOR SEQ ID NO: 67:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- 15 (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

20 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 67:

25

Tyr Asp Ile Ala Lys Ile Ser Gln His

1

5

30 (2) INFORMATION FOR SEQ ID NO: 68:

(i) SEQUENCE CHARACTERISTICS:

69

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 68:

Leu Asp Phe Ile Ser Ile Met Thr Tyr

15 1 5

(2) INFORMATION FOR SEQ ID NO: 69:

20 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

25

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

30

70

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 69:

Phe Ile Ser Ile Met Thr Tyr Asp Phe

1 5

5

(2) INFORMATION FOR SEQ ID NO: 70:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 70:

Phe Arg Gly Gln Glu Asp Ala Ser Pro

1 5

25

(2) INFORMATION FOR SEQ ID NO: 71:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

71

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

5 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 71:

10

Tyr Ala Val Gly Tyr Met Leu Arg Leu
1 5

15 (2) INFORMATION FOR SEQ ID NO: 72:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- 20 (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

25 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 72:

30

Met Leu Arg Leu Gly Ala Pro Ala Ser
1 5

(2) INFORMATION FOR SEQ ID NO: 73:

5 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 73:

Leu Ala Tyr Tyr Glu Ile Cys Asp Phe

1 5

20

(2) INFORMATION FOR SEQ ID NO: 74:

25 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 74:

5

Leu Arg Gly Ala Thr Val His Arg Thr

1

5

10 (2) INFORMATION FOR SEQ ID NO: 75:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids

(B) TYPE: amino acid

15

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

20

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 75:

25

Tyr Leu Lys Asp Arg Gln Leu Ala Gly

1

5

30 (2) INFORMATION FOR SEQ ID NO: 76:

(i) SEQUENCE CHARACTERISTICS:

74

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 76:

Leu Ala Gly Ala Met Val Trp Ala Leu

15 1

5

(2) INFORMATION FOR SEQ ID NO: 77:

20

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

25

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 77:

Val Trp Ala Leu Asp Leu Asp Asp Phe

1 5

5

(2) INFORMATION FOR SEQ ID NO: 78:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- 10 (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

15

(v) FRAGMENT TYPE: internal

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 78:

Leu Asp Leu Asp Asp Phe Gln Gly Ser

1 5

CLAIMS

1. Peptides consisting of 16 to 55 amino acid residues, said peptide comprising at least one of the amino acid sequences LVCYYTSWS (SEQ ID NO:60), FLCTHIIYS (SEQ ID NO:61), IIYSFANIS (SEQ ID NO:62), LKTLLSVGG (SEQ ID NO:63), FIKSVPPL (SEQ ID NO:64), FDGLDLAWL (SEQ ID NO:65), LYPGRRDKQ (SEQ ID NO:66), YDIAKISQH (SEQ ID NO:67), LDFISIMTY (SEQ ID NO:68), FISIMTYDF (SEQ ID NO:69),
5 FRGQEDASP (SEQ ID NO:70), YAVGYMLRL (SEQ ID NO:71), MLRLGAPAS (SEQ ID NO:72), LAYYEICDF (SEQ ID NO:73), LRGATVHRT (SEQ ID NO:74), YLKDRQLAG (SEQ ID NO:75),
10 LAGAMVWAL (SEQ ID NO:76), VWALDLDDF (SEQ ID NO:77) or LDLDLDFQGS (SEQ ID NO:78).
- 15 2. Peptide consisting of 16 to 55 amino acid residues, said peptide comprising at least one of the amino acid sequences YKLCYYTSWSQYREG (SEQ ID NO:1), YTSWSQYREGDGSCFP (SEQ ID NO:2), LDRFLCTHIIYSFANI (SEQ ID NO:5), THIIYSFANISNDHID (SEQ ID NO:6), PNLKTLLSVGGWNFGS (SEQ ID NO:12), NTQSRRTFIKSVPPFL (SEQ ID NO:16), TFIKSVPPLRTHGFD (SEQ ID NO:17), PPFLRTHGFDGLDLAW (SEQ ID NO:18), HGFDGLDLAWLYPGRR (SEQ ID NO:19), DLAWLYPGRRDKQHFT (SEQ ID NO:20), TIDSSYDIAKISQHLD (SEQ ID NO:28), DIAKISQHLDLDFISIMT (SEQ ID NO:29), QHLDLDFISIMTYDFHGA (SEQ ID NO:30),
20 SPLFRGQEDASPDRFS (SEQ ID NO:34), DYAVGYMLRLGAPASK (SEQ ID NO:37), MLRLGAPASKLVMGIP (SEQ ID NO:38), PASKLVMGIPTFGRSF (SEQ ID NO:39), GTLAYYEICDFLRGAT (SEQ ID NO:46), EICDFLRGATVHRTL (SEQ ID NO:47), RGATVHRTLQQQVPA (SEQ ID NO:48), VKSKVQYLKDRQLAGA (SEQ ID NO:53), YLKDRQLAGAMVWALD
25 (SEQ ID NO:54), LAGAMVWALDLDDFQG (SEQ ID NO:55),
- 30

WALDLDDFQGSFCGQD (SEQ ID NO:56) or DFQGSFCGQDLRFPLT (SEQ ID NO:57).

3. Peptide according to claim 1 or 2, said peptide comprising at least one of the amino acid sequences YKLVCYYTSWSQYREG (SEQ ID NO:1), YTWSQYREGDGSCFP (SEQ ID NO:2), LDRFLCTHIIYSFANI (SEQ ID NO:5), THIIYSFANISNDHID (SEQ ID NO:6), PNLKTLLSVGGWNFGS (SEQ ID NO:12), QHLDLFISIMTYDFHGA (SEQ ID NO:30), SPLFRGQEDASPDRFS (SEQ ID NO:34), DYAVGYMLRLGAPASK (SEQ ID NO:37), MLRLGAPASKLVMGIP (SEQ ID NO:38), YLKDRQLAGAMVWALD (SEQ ID NO:54) or LAGAMVWALDLDDFQG (SEQ ID NO:55).
4. Peptide according to any of claims 1 to 3, said peptide comprising at least one of the amino acid sequences YTWSQYREGDGSCFP (SEQ ID NO:2), SPLFRGQEDASPDRFS (SEQ ID NO:34), MLRLGAPASKLVMGIP (SEQ ID NO:38), YLKDRQLAGAMVWALD (SEQ ID NO:54) or LAGAMVWALDLDDFQG (SEQ ID NO:55).
5. Hexadecapeptide according to claim 1 to 4, said hexadecapeptide consisting of one of the amino acid sequences YKLVCYYTSWSQYREG (SEQ ID NO:1) YTWSQYREGDGSCFP (SEQ ID NO:2), LDRFLCTHIIYSFANI (SEQ ID NO:5), THIIYSFANISNDHID (SEQ ID NO:6), PNLKTLLSVGGWNFGS (SEQ ID NO:12), QHLDLFISIMTYDFHGA (SEQ ID NO:30), SPLFRGQEDASPDRFS (SEQ ID NO:34), DYAVGYMLRLGAPASK (SEQ ID NO:37), MLRLGAPASKLVMGIP (SEQ ID NO:38), YLKDRQLAGAMVWALD (SEQ ID NO:54) or LAGAMVWALDLDDFQG (SEQ ID NO:55).
6. Peptide according to any of the claims 1 to 5 for use as a medical substance.

7. Pharmaceutical composition comprising one or more peptides according to any of the claims 1 to 5, and a pharmaceutical acceptable carrier.
8. A diagnostic method for the detection of activated autoreactive T cells comprising the following steps: -a) isolation of the peripheral blood mononuclear cells (PBMC) from a blood sample of an individual, b) culture of said PBMC under suitable conditions, c) incubation of said PBMC culture in the presence of one or more peptides according to any of the claims 1 to 5, and d) detection of a response of T cells, for example a proliferative response, indicating the presence of activated autoreactive T cells in the individual.
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9. Testkit, for the detection of activated autoreactive T cells, said test kit comprising one or more peptides according to any of the claims 1 to 5.
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 97/02051

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 C07K14/47 A61K38/17 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 IPC 6 C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 01995 A (THE REGENTS OF UNIVERSITY OF CALIFORNIA) 19 January 1995 see the whole document ---	1-9
X	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 268, no. 34, 5 December 1993, MD US, pages 25803-25810, XP002014036 B E HAKALA ET AL.: "Human cartilage gp-39, a major secretory product of articular chondrocytes and synovial cells, is a mammalian member of chitinase protein family" cited in the application *the whole disclosure, especially Table 1* ---	1,2,6,7

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- 'A' document defining the general state of the art which is not considered to be of particular relevance
- 'E' earlier document but published on or after the international filing date
- 'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- 'O' document referring to an oral disclosure, use, exhibition or other means
- 'P' document published prior to the international filing date but later than the priority date claimed

- 'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- 'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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- '&' document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
25 August 1997	09.09.97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Authorized officer Masturzo, P

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 97/02051

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BIOCHEMICAL JOURNAL, vol. 269, no. 1, 1 July 1990, LONDON, pages 265-268, XP002014037 P NYIRKOS & E GOLDS: "Human synovial cells secrete a 39 kD protein similar to a bovine mammary protein expressed during the non-lactating period" *the whole disclosure, especially Figure 3* --- T WO 96 13517 A (AKZO NOBEL NV) 9 May 1996 see the whole document -----	1,2,6,7 1-9
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INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat'l Application No

PCT/EP 97/02051

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